3. The "incidence" of a sound is hence determined by its timbre and loudness. Experimentally produced changes in the timbre or loudness of a sound lead to marked changes in its apparent incidence.

4. Tactual sensibility appears to play no part in auditory localisation. Localised tactual sensations evoked by auditory stimuli are generally the outcome of interpretations by the subject, resulting from his natural tendency to treat sounds as material objects, and to refer to them a localisation based on solely auditory data.

A Comparative Study of Oxidation by Catalysts of Organic and Inorganic Origin.

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CONTENTS.

The present paper is the outcome of the work carried out on the influence of poisoning on apples and potatoes, and its progress has necessitated a

general revision of the oxidase ferments, and in particular a general comparison with metallic oxidases. As is well known, oxidases are widely distributed in plants, and are frequently responsible for the changes of colour in extracted plant juices or in plant tissues after death.

In the case of the apple and potato, the curious fact that browning took place in pulp killed by immersion in poisonous metallic solutions, but not when killed by heat, demanded special investigation.

The Browning of Apples and Potatoes.

It is generally assumed that this is due to the action of an oxidase ferment upon a chromogen present in the pulp cells, such as tannic acid in the apple* and tyrosin in the potato, but the oxidase is not necessarily the same in either case. The term "oxidase" in fact rather represents a result than a particular substance, and many "oxidase" actions are not necessarily due to organised ferments or enzymes at all. In a previous paper it has been shown that apple pulp immersed in solutions of metallic poisons may still turn brown, although ordinary ferments are destroyed by such poisons. Furthermore, the reasons why the browning takes place on death, but not in the living cell, and not when the cell is killed in certain special ways need investigation. Pro-chromogens or zymogens may exist in the living cell which decompose into interacting chromogen and enzyme on death, or the latter may be kept apart in the living cell by semipermeable membranes which lose their impermeability on death. In the latter case the localisation of the chromogen and enzyme in the cell becomes a problem of special importance.

According to Gruss† "Antioxidases"‡ capable of arresting oxidase reactions exist in various plants, and if the oxidase and the "antioxidase" balanced, a chromogen and its oxidase might exist in contact in the living cell and the mode of death might determine whether browning occurred or not. Behrens§ considered that the browning of the apple pulp is due to a direct oxidation of tannic acid to form a leathery compound with the proteids of the cell, without the aid of an oxidase.

The first point needing full investigation was the influence of poisons on browning, particularly in regard to the time factor and the rapidity of penetration.

* Lindet, 'Compt. Rend.,' vol. 120, p. 370 (1895).

† 'Biologie und Capillaranalyse der Enzymen,' p. 56 (1912).

‡ To avoid possible confusion, the word "inhibitor" may be used instead of this term.

§ 'Centralbl. f. Bakt.,' 2 Abth., vol. 4, p. 514 (1898).

The Influence of Poisons on Browning.

As is well known, apple and potato pulp if killed by dropping into boiling water, remains colourless in the presence of oxygen for an indefinite length of time, and this according to Bourquelot is due, in the case of the apple, to the destruction of the oxidase responsible for browning. When portions of apple pulp are immersed in very dilute sulphuric or tartaric acids, the pulp turns brown, whereas in stronger solutions it remains colourless. This might be due to the stronger acid inhibiting or destroying the oxidase ferment, or preventing its formation if only present as a zymogen in the living cell.

Pulp pounded in its own volume of 1-per-cent. H_2SO_4 remains colourless and gives no guaiacum test and no distinct decomposition of H_2O_2. If pounded and allowed to brown before adding the sulphuric acid, no oxidase reactions are shown after, but active ones before the addition of the acid. Slices of fresh pulp decompose H_2O_2 actively and also turn guaiacum blue. If pulp pounded with 10-per-cent. H_2SO_4 is neutralised with ammonia and tested, it gives no oxidase reactions. Apparently, therefore, the sulphuric acid acts directly by destroying the oxidase present in the living cells.

Pieces of apple pulp immersed in poisonous metallic solutions develop a brown colour on drying, and the same is shown whatever the concentration. In 1 and 5-per-cent. solutions of lead nitrate, however, the browning is fainter than usual and is mainly confined to the veins. Lead nitrate destroys oxidase ferments and hence apparently the production of browning and the presence of oxidase are not exactly parallel. The addition of dilute ammonia rapidly turns the pulp a deeper brown, but the immediate addition of dilute H_2SO_4 or HCl restores the original pale colour. Hence the browning produced by ammonia is not quite the same as the permanent brown produced in slowly dying pulp cells. If the pulp is soaked in dilute ammonia for some hours, acids will not, however, entirely remove the brown colour.

When pieces of pulp are soaked in a poisonous solution, a certain time elapses between the first penetration of poison and the death of each cell, and this time-interval will be greater in the case of deeply seated cells than of superficial ones. This is well shown when prepared potatoes are immersed in 5 or 10-per-cent. solutions of lead nitrate. Chromogen oxidation only takes place towards the inner boundary of the diffusion zone.

To eliminate the time factor the pulp was rapidly pounded in a mortar with the poisonous solution and then tested with guaiacum and H_2O_2. French crab apples were used.

Poison.	Colour change in air.	Guaiacum test.	Decomposition of H_2O_2.
Untreated	Brown	Blue	Active.
1 per cent. $HgCl_2$	None	Pale blue	Feeble.
1 „ $CuSO_4$.....	„	Deep blue	Strong.
1 „ Pb_2NO_3	„	No blue	None.
5 „ Pb_2Ac	Pale greenish or yellowish brown	Pale blue	Fairly active.
1 „ $AgNO_3$	Blackens	Strong blue especially with H_2O_2	None.
1 „ morphine sulphate	Brown	Pale blue	Feeble.
10 „ strychnine	„	„	„
10 „ brucin nitrate ...	„	„	„
2 „ $BaClO_3$	None	„	„
2 „ $FeCl_3$	Rapidly changing to dark brown	Deep blue	Active.
Absolute alcohol	None	Faint blue	Doubtful.
Boiled pulp	Nil	Nil	Very feeble.

With fresh juice or pulp from potatoes the following results were obtained :—

Poison.	Colour change in air.	Guaiacum test.	Decomposition of H_2O_2.
5 per cent. lead nitrate	Nil	Nil	Nil.
5 „ mercuric chloride	„	Blue	Very feeble.
5 „ copper sulphate ...	„	Deep blue	Active.
Untreated	Brown	Strong blue	Active.
Boiled	Nil	Nil	Very feeble.

At first sight these results seem to show that browning is not closely related with the presence of the oxidase. With absolute alcohol, however, if the pulp is allowed to stand for a short time the oxidase reactions entirely disappear. Apparently the alcohol first weakens and then destroys the oxidase and the oxidation of tannic acid seems to require a more powerful oxidase action than is necessary to produce a blue with guaiacum.

Inorganic Oxidases.

In addition the influence of the metallic poisons must be taken into account. According to L. Meyer,* salts of manganese such as the chloride and sulphate can act as strong oxidases, and salts of copper, iron, and cobalt have the same power but progressively decreasing, whilst the least oxidase action is shown by salts of nickel, zinc, cadmium, and magnesium. It is not, however, clear as to whether a strong metallic oxidase oxidises all substances

* 'Ber. Chem. Gesell.,' vol. 20, p. 3085 (1887).

capable of oxidation with the same relatively greater intensity than does a feeble oxidase, or whether a strong oxidase to one substance may be a feeble oxidase to another as in the case of the organic oxidases. Other points needing elucidation are as to whether oxidase action is solely due to the metallic base, and what influence a metal such as iron will have when present as an acid. Also as to whether peroxide of hydrogen only accelerates oxidase action when the oxidase salt decomposes it. In the following table the results of a series of tests are given using guaiacum, ursol tartrate,* hydroquinone, pyrogallic acid, gallic acid, tannic acid, and tyrosin as oxidant substances. In order to render possible a comparison of the relative activities in each case, the oxidase was present in one-tenth the molecular concentration of the oxidant, except in the case of the guaiacum, where the alcoholic solution is best allowed to float as a thin layer on the oxidase solution.

The exposure to air was continued for one day, and if the colour was still the same as the test solution the result is given as nil. A rapid reaction is indicated by three positive signs (+ + +), slower ones taking one or more hours to become distinctly perceptible by two signs (+ +), a very slow one taking the full 24 hours by one (+). In the case of guaiacum, owing to the mode of application of the test, the time factor does not enter to the same extent, but a difference in the strength of the oxidase is indicated by the depth of the blue coloration (strong = + + +, weaker blue = + +, feeble blue = +).

	Katalase action.	Guaiacum.	Ursol tartrate.	Pyrogallol.	Hydro-quinone.	Gallic acid.	Gallotannic acid.	Tyrosin.
Cupric chloride.....		+ +		+		+		
Do. + H_2O_2 ...	+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+	
Cupric sulphate ...							+	
Do. + H_2O_2 ...		+ +	+	+ + +	+ + +	+ + +	+	
Copper oxychloride				+				
Do. + H_2O_2 ...		+	+	+ +	+ +	+ + +	+	+
Copper acetate and subacetate		+		+	+			
Do. + H_2O_2 ...	+*	+ + +	+	+ +	+ +	+ +	+	+
Ferric chloride		+ + +	+ + +	+ +				
Do. + H_2O_2 ...	+ + +	+ + +	+ + +	+ + +	+ + +†	+ +?	+?	+
Ferrous sulphate ...								
Do. + H_2O_2 ...	+ + +‡	+ +	+ +	+ +	+ +	+ +?	+?	+

* $Cu_3O_2Cl_2,4H_2O$.

† Flat black shining needles separate out on standing in the cold, but no distinct oxidase action is shown.

‡ Liquid yellow, then slowly brown precipitate.

* Paraphenylenediamine tartrate.

	Katalase action.	Guaiacum.	Ursol tartrate.	Pyrogallol.	Hydro-quinone.	Gallic acid.	Gallotannic acid.	Tyrosin.
Ferrous chloride ...		+						
Do. + H_2O_2 ...	+ + +*	+ +	+ +	+ +	+ +	+ +	+	+
Potassium ferro-cyanide								
Do. + H_2O_2 ...	+†	+ + +	+ +	+ +	+	+ +		
Potassium ferri-cyanide		+	+ +	+ +		+		
Do. + H_2O_2 ...		+ + +	+ + +	+ + +	+ +	+ +		
Manganese chloride ($MnCl_2$)				+				
Do. + H_2O_2 ...		+?	+	+ +	+	+		
Manganese sulphate ($MnSO_4$)								
Do. + H_2O_2 ...		+ +	+ +	+	+	+		
Potassium permanganate		+ + +	+ + +	+	+	+ +		+ +
Do. + H_2O_2 ...	+ + +	+ + +	+ + +	+ +	+ +	+	+	
Black oxide of manganese		+ +	+ + +	+ + +	+	+ +		
Do. + H_2O_2 ...	+ + +	+ + +	+ + +	+ +	+ +	+		
Chromium chloride								
Do. + H_2O_2 ...	Nil	Nil	+ +	+ +	+ +	+	+	+
Chromic acid		+ + +	+ +	+ + +	+ +	+ + +	+ + +	
Do. + H_2O_2‡	+ + +	+ + +	+ + +	+ + +	+ +	+ +	+ +	+
Potassium bichromate		+ + +	+ +	+ +	+	+ + +		
Do. + H_2O_2‡	+ + +	+ + +	+ + +	+ + +	+ +	+ +	+ + +	+
"Neutral" potassium phosphate§				+				
Do. + H_2O_2 ...	Nil	Nil	+	+	+			
Nitric acid		+ + +	+	+ + +	+ +	+ +	+	
Do. + H_2O_2 ...				+ +		+		
Lead acetate								
Do. + H_2O_2 ...	+	+ + +	+	+ +	+	+	+	

* Ferric salt formed, hence the oxidase reactions with H_2O_2 the same as with ferric chloride.

† H_2O_2 converts ferrocyanide partly into ferricyanide. Hence ferrocyanide and H_2O_2 give similar oxidase reactions to the ferricyanide. Similarly traces of ferricyanide appear slowly in a solution of ferrocyanide exposed to light and the liquid becomes a deeper yellow, while ultimately prussian blue separates out. According to Sarthou ('Journ. Pharm. Chim.,' vol. 1, p. 482, 1900), the bark of *Schinus molle* contains a ferment "schinoxidase" which converts potassium ferrocyanide into ferricyanide. It is, however, very doubtful in this case that we are dealing with an oxidase ferment at all.

‡ With dilute solutions the colour change with hydroxyl is easily distinguished from the oxidase change by using controls. A mixture of dilute CrO_3 and H_2O_2 becomes colourless again on long standing.

§ Made by adding potassium carbonate to a boiling solution of acid potassium phosphate until imperceptibly acid or alkaline to litmus.

The foregoing Table shows clearly that an inorganic oxidase is not necessarily a "katalase," nor a katalase an oxidase, and that hydrogen peroxide may accelerate the oxidase action of substances incapable of decomposing it.

In the case of nitric acid, chromic acid and potassium permanganate the

oxidation is, in part at least, a direct one and hydrogen peroxide diminishes the oxidising action. If hydrogen peroxide is added to very dilute potassium permanganate, a colourless liquid is formed and the evolution of oxygen soon ceases. With stronger solutions the liquid is brown, and contains an oxide capable of continuous oxidase and katalase action.

The Tables show further that chromium and iron can act as oxidases when present in the form of acids.

In general a feeble oxidase acts feebly on all the substances tested, and the order of sensitivity to oxidases is: guaiacum, ursol tartrate, pyrogallol, hydroquinone, gallic acid, gallotannic acid, tyrosin. There are, however, various exceptions. Thus chromium chloride and manganese chloride give a blue with guaiacum, and copper sulphate does the same in the presence of H_2O_2, but all three give direct oxidase reactions with ursol tartrate, pyrogallol, etc. Where there is a strong tendency to precipitation between the oxidase and oxidant as in the case of lead acetate or of ferric chloride and hydroquinone, the oxidase action may be retarded or prevented. In the case of potassium phosphate the feeble oxidase properties are evidently due to the phosphoric acid and not to the potassium. Further, the chlorides, nitrates or sulphates of the same metal are not necessarily equally powerful oxidases, chlorides apparently surpassing sulphates (see copper) and nitrates chlorides. Thus cobalt chloride shows no oxidase properties with or without H_2O_2. Cobalt nitrate slowly browns pyrogallol and hydroquinone in the presence of H_2O_2 but is inactive to guaiacum, ursol tartrate, and tannic acid.

Lead nitrate shows no oxidase action, whereas lead acetate exhibits a peroxidase action. Yellow potassium chromate has similar oxidase properties to potassium bichromate except that it causes tannic acid to brown rapidly in the absence of H_2O_2, probably owing to the alkaline nature of the basic chromate.

The Nature of Oxidases.

The fact that certain plant oxidases contain oxidase metals, such as manganese in laccase, has long been known and certain oxidases, as for instance, tobacco oxidase, can be boiled without being destroyed. Woods* considers that this is due to the oxidase existing as a zymogen from which on cooling the oxidase is reproduced. The supply of zymogen can, however, hardly be unlimited, and, since the boiling can be repeated more than once without destroying the oxidase, it must itself be resistant to heat.

According to Bach and Chodat† the oxidases form three distinct groups of ferments, namely :—

* 'Bull. U.S. Dept. Agric.,' vol. 18, p. 17.

† 'Biochem. Centralbl.,' 1903, p. 141.

(1) Oxygenases, proteins which absorb molecular oxygen forming peroxides;

(2) Peroxidases, which increase the oxidising power of peroxides and can only act in their presence;

(3) Katalases, which destroy peroxides with an evolution of oxygen.

An oxidase which turns guaiacum blue without hydrogen peroxide being added is a mixture of ferments of the first and second class. Thus Bach* considers tyrosinase to be a mixture of a specific oxidase and a specific peroxidase.

Moore and Whitley† conclude that all oxidases are peroxidases acting in the presence of a peroxide, such as may be present in certain solutions of guaiacum, or an organic peroxide derived from the juice of the plant tested. The peroxides can be removed from the juices and from guaiacum by adding animal charcoal and filtering, and they are destroyed by heating to 55°–60° C. for several hours. After such treatment potato juice will only give a blue with guaiacum on adding H_2O_2.

As a matter of fact the action is merely to attenuate the oxidase, so that the addition of an accelerator such as H_2O_2 is necessary to render the oxidation of the guaiacum perceptible. The facts that potato juice decomposes peroxides strongly, and that the juice and pulp give negative results when tested with decolorised magenta, and show no effervescence until H_2O_2 is added, are hardly in accord with Moore and Whitley's explanation, and a study of the preceding Tables shows that, so far as metallic oxidases are concerned, too much importance can easily be attached to the influence of peroxide of hydrogen on oxidase action. Thus in some cases the same substance may be a katalase, an oxidase, and a peroxidase. In other cases the same metallic salt may be an oxidase to one reagent, a peroxidase to another, ineffective to another, and may or may not at the same time be a katalase. Finally, the mere addition of H_2O_2 may convert a weak oxidase ("peroxidase") into a stronger one, which will then act in the absence of H_2O_2 (ferrous salts and potassium ferrocyanide).

Moore and Whitley found that hydrochloric acid of 1/800 normal concentration nearly destroyed potato oxidase, and quite destroyed carrot oxidase, while sodium hydrate of similar concentration had no effect. On the other hand, Na_2HPO_4 had a stronger destructive action than NaH_2PO_4. The reaction may, however, be prevented without the oxidase being destroyed. Thus the addition of hydrochloric acid (or sulphuric) to ferric chloride removes its power of giving a blue with guaiacum, while tartaric and citric

* 'Berichte,' vol. 39, No. 10, p. 2126 (1906).

† 'Biochem. Journ.,' vol. 4, p. 136 (1909).

acids hinder or decrease the blue reaction, which is however given strongly even in the presence of 1-per-cent. oxalic acid. Hydrogen peroxide is partially antagonistic to this action, and it is possible to obtain acidified solutions of ferric chloride which will give a blue when peroxide of hydrogen has been added, but not when it is absent. The effect probably depends upon the ionic condition of the iron in solution, but the disappearance of an oxidase reaction on the addition of acid does not necessarily mean that the oxidase has been destroyed, any more than ferric chloride is destroyed by the addition of hydrochloric acid. Further, in Moore and Whitley's experiment with expressed potato sap heated to 55° C. for some hours, the sap becomes more acid, and this might in itself be a sufficient explanation of why an addition of peroxide of hydrogen then becomes necessary to obtain an oxidase reaction.

Further, the case of cobalt chloride and ammonia shows that the addition of alkali to certain plant oxidases might greatly increase their oxidase activity or might convert a non-oxidase combination into an oxidase one. Thus, with a small quantity of ammonia, cobalt chloride forms a green precipitate, slowly oxidising to brown; but with slight access of ammonia a nearly colourless liquid is formed, oxidising to brown from the surface. If the two liquids are diluted, the latter gives a blue with guaiacum directly, and the former an intense blue on adding H_2O_2; but without H_2O_2 no blue is given, or only a faint trace on long standing. In other words, an oxidisable substance can act as an oxidase, and ammonia, by accelerating the rate of auto-oxidation, also increases the intensity of oxidase action and converts a peroxidase into an oxidase.

According to Porodko,* *per* salts (-ic) of iron, copper, manganese, and chromium give a blue with guaiacum in the absence of peroxide of hydrogen, *proto* salts (-ous) only when it is present. This is, however, by no means a general rule. Ferrous chloride gives a blue without H_2O_2, but not cupric sulphate or manganic chloride in moderate dilution. Lead nitrate gives no blue in the presence or absence of hydrogen peroxide, while lead acetate gives a strong blue in its presence. Further, the addition of H_2O_2 converts the non-oxidase ferrous sulphate or potassium ferrocyanide into ferric compounds, which give a blue with guaiacum in the absence of hydrogen peroxide.

Oxidase Sensitisers and Inhibitors.

Various neutral salts may exert a powerful action on metallic oxidases either as sensitisers or retardants. Thus an old test for a soluble copper salt given by Purgotti is: Add salt and pour on top an alcoholic solution of

* 'Bot. Centralbl.,' Beihefte 2, vol. 16, p. 1 (1904).

guaiacum—strong blue colour. Either sodium or potassium chloride will cause copper sulphate to give as deep a blue as in the presence of H_2O_2, *i.e.* converts it from a "peroxidase" to an oxidase. The action is not solely due to cupric chloride being present in the mixed solution, since the blue is deeper than with cupric chloride alone. The intensity of the action decreases slowly with increasing dilution. It is given by a dilution of 1 grm. of copper sulphate in 100,000 c.c. of water, faintly in a dilution of 1 in 250,000, very faintly by 1 in 100,000, and not at all in a solution of 1 in 10,000,000; *i.e.* guaiacum is about as sensitive in the presence of salt to the oxidase action of copper sulphate as the pulp cells of apples are to its poisonous action.

Sodium and potassium phosphates are also able to act as sensitisers to such oxidases as potassium ferricyanide, and the influence of a sensitiser may show with some but not necessarily with all test substances (oxidants). The accelerating action of phosphates is particularly marked with tannic acid, if sufficient is added to leave a clear solution. An excess produces a purplish white precipitate and naturally interferes with oxidation. Acid potassium phosphate is a less active oxidase sensitiser than the neutralised solution of the same salt. Neutral potassium phosphate accelerates the oxidation of tannic acid by potassium permanganate, but not by black oxide of manganese, and it acts as a retardant to those soluble metallic oxidases which it precipitates.

Water itself may act as a sensitiser as well as an oxygen carrier. Thus, if potassium ferricyanide is dissolved in pure boiled glycerine and guaiacum dissolved in absolute alcohol added, even after long standing only a faint blue or none at all appears at the junction of the two liquids, which rapidly intensifies on adding a little water. Nasse and Fram* have even gone so far as to ascribe the oxidation entirely to the hydroxidation of water without the presence of free oxygen being necessary, but Porodko† has shown that this is not the case.

The addition of a neutral solution of potassium phosphate to potassium ferrocyanide does not cause any ferricyanide to appear, but causes it to behave as a weak oxidase to guaiacum, ursol tartrate, pyrogallol and hydroquinone, and as a "peroxidase" to gallic acid, tannic acid and tyrosin. The action in the presence of hydrogen peroxide is, however, in part due to its partial conversion into ferricyanide. Neutral potassium phosphate intensifies the oxidase reaction of potassium ferricyanide and converts it from a non-oxidase to tannic acid and tyrosin into an oxidase to the former and a "peroxidase" to the latter.

* 'Pflüger's Archiv,' vol. 63, p. 203 (1896).

† 'Bot. Centralbl.,' Beihefte, vol. 10, p. 1 (1904).

	Katalase action.	Guaiacum.	Ursol tartrate.	Pyrogallol.	Hydro-quinone.	Gallic acid.	Gallotannic acid.	Tyrosin.
Acid potassium phosphate								
Do. $+H_2O_2$	+		+					
Neutral potassium phosphate				?				
Do. $+H_2O_2$			+	+	+			
Potassium ferrocyanide and acid phosphate						?	?	
Do. $+H_2O_2$	+	+ + +	+ +	+		+	+	*
Potassium ferrocyanide and neutral phosphate		+	+ +	+				
Do. $+H_2O_2$	+	+ +	+ + +	+ +	+ +	+ +	+ +	+
Potassium ferricyanide and acid phosphate		+ +	+	+	?	+ +	+ +	
Do. $+H_2O_2$		+ + +	+ + +	+	+	+	+ +	
Potassium ferricyanide and neutral phosphate		+ + +	+ + +	+ +	+	+ + +	+ + +	
Do. $+H_2O_2$	+	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+

* Yellowish colour due to formation of ferricyanide.

To some extent acid potassium phosphate and peroxide of hydrogen are antagonistic in their action on potassium ferricyanide, and hence the oxidation produced when both are added may be no greater or even less than when either is added singly.

In the case of tannic acid, the addition of sodium or potassium phosphate seems not so much to accelerate the action of the oxidase as to render the tannic acid more liable to oxidation. The chlorides and bromides of sodium and potassium act as strong sensitisers to certain metallic oxidases but not to others, and even with the former the sensitising action is not the same to all oxidants. By themselves these salts exhibit no oxidase properties with any of the oxidants mentioned.

A detailed comparison is given beneath of the influence of potassium chloride upon the oxidase action of relatively inert oxidase salts such as

	Katalase action.	Guaiacum.	Ursol tartrate.	Pyrogallol.	Hydro-quinone.	Gallic acid.	Tannic acid.	Tyrosin.
Copper sulphate and sodium chloride	+ +	+ + +		+		+	+	
Ferrous sulphate and potassium chloride	+ +	+*		+		†		
Chromium chloride and sodium chloride		+		+				

* Fading again on long standing.
† A pale violet colour darkening from the surface possibly owing to oxidation to ferric salts.

copper sulphate, ferrous sulphate and chromium chloride, which act as oxidases in the presence of hydrogen peroxide but not in its absence.

Copper sulphate, in the presence of salt and H_2O_2, rapidly causes an oxidase browning in tannic acid, a slight browning is slowly produced with tyrosin, and a full colour sequence with ursol tartrate. The oxidase reactions of copper sulphate and H_2O_2 with pyrogallol, hydroquinone, and gallic acid are approximately the same in the presence as in the absence of salt.

In the presence of H_2O_2 salt slightly accelerates the oxidase action of chromium chloride on hydroquinone and pyrogallol, and a deep blue is given with guaiacum, particularly if bromide is used instead of chloride, but a weaker blue if no chloride or bromide is present. In general sodium and potassium bromides are slightly stronger sensitisers than the chlorides, the iodides are less active* and the fluorides still more so or may even exercise the reverse action.

Copper acetate, ferrous chloride and potassium ferricyanide, which slowly give a pale blue with guaiacum in the absence of H_2O_2, give a stronger blue rapidly in the presence of salt nearly as well as when H_2O_2 is added. Salt is, however, unable to produce a blue in the absence of H_2O_2 with manganese sulphate or chloride, with copper oxychloride or with potassium ferrocyanide.

The relative mass of the oxidase and sensitiser is of importance. Thus if equal masses of ferrous sulphate and of KCl, KI, or KF are present, and the solutions fairly strong, no blue is given with guaiacum, but if the ferrous sulphate is present in relatively dilute solution, a rather pale blue is given with KCl, weaker with KBr and KI, and faint or imperceptible with KF. Hence if the oxidase and sensitiser are not present in the proper proportions some oxidase actions may be prevented or overlooked. Copper sulphate, however, even when present in excess gives direct oxidase reactions in the presence of sensitisers, possibly because unlike ferrous sulphate it has no tendency to auto-oxidation. On the other hand, a sensitiser does not act with all oxidase tests. Thus neither KI, KBr, KCl nor KF, whether relatively dilute or concentrated, give ferrous sulphate any oxidase action on ursol tartrate or hydroquinone.

The double fluoride of sodium (NaFHF) inhibits the oxidase action of ferric chloride on guaiacum, ursol tartrate, pyrogallol, hydroquinone and tyrosin, and also its power of decomposing H_2O_2. It strongly retards the oxidase action of potassium ferricyanide and of manganese sulphate and H_2O_2, and although a blue is still given with guaiacum it is much paler. A rather weaker retarding action is also exercised upon the oxidising action of potassium permanganate

* They cannot be tried with copper, owing to the precipitation of the latter, or used in the presence of H_2O_2, owing to the decomposition of the iodide by the peroxide.

and upon its power of decomposing H_2O_2, the solution in the latter case remaining clear instead of turning brown.

The single fluoride (NaF) is also capable of acting as an antagoniser, particularly shown in the case of ferric chloride with guaiacum and hydroquinone, while with pyrogallol a violet-blue is given instead of dark brown. On the other hand the addition of sodium fluoride to potassium ferricyanide increases all its oxidase reactions except to pyrogallol, where a retardation is shown, and renders it a weak oxidase to tannic acid and a "peroxidase" to hydroquinone.

If a dilute solution of lead nitrate is added to a dilute solution of copper acetate and salt in such proportion that two atoms of chlorine are present to each atom of lead, a pale blue is still given with guaiacum, but none if the lead nitrate is in excess, although in neither case is any precipitate formed. Lead nitrate might, therefore, be regarded as an antagoniser to copper acetate and sodium chloride as a sensitiser. An excess of lead nitrate prevents copper acetate giving a distinct blue by itself, but a blue is still given in the presence of H_2O_2.

In the case of potassium permanganate the addition of sodium fluoride does not affect the guaiacum test or the decomposition of H_2O_2, slightly accelerates the oxidation of tannic acid (in the presence of H_2O_2) and of ursol tartrate, and distinctly retards the oxidation of pyrogallol and hydroquinone. The same substance may, therefore, be a sensitiser to one oxidase and an antagoniser to another, while the action may vary according to the substance oxidised.

Sensitisers and Antagonisers to Plant Oxidases.

It is well known that diastase acts as an oxidase to guaiacum and the same applies to other ferments. Plant oxidases, however, appear to be more specific and less generalised in their action than are metallic oxidases. Hence it is of importance to determine to what extent the specific peculiarities of certain plant oxidases can be ascribed to the presence of accompanying sensitisers and antagonisers or inhibitors.

In the following Table a general comparison is given between a few of the common plant ferments and oxidases. In the first four cases watery solutions were used, in the last two cases thin slices of potato and apple were rapidly dried *in vacuo* after squeezing out the sap and were pounded to powder. The powder was added to the test solution.

The chief peculiarities are that potato oxidase acts strongly on tyrosin and feebly or not at all on tannic acid or on ursol tartrate in the absence of hydrogen peroxide, while apple oxidase, which is generally weaker, acts strongly on tannic acid and ursol tartrate but not at all on tyrosin.

	Katalase action.	Guaiacum.	Ursol tartrate.	Pyrogallol.	Gallic acid.	Tannic acid.	Tyrosin.
Malt diastase		+					
Do. + H_2O_2	+	+ + +	+	+			
Papain				+			
Do. + H_2O_2		+	+	+			
Pepsina porci							
Do. + H_2O_2		+					
Pancreatin							
Do. + H_2O_2							
Potato powder		+ + +		+			
Do. + H_2O_2	+ + +	+ + +	+ +	+ + +	+ +		
Apple powder		+ + +	+ +	+		+	
Do. + H_2O_2	+ +	+ + +	+ + +	+ +	+ +	+ +	

Neither the chlorides nor the phosphates of potassium or sodium accelerate the oxidase action of malt diastase. If anything the addition of malt diastase to potassium phosphate appears to slightly retard the feeble oxidase action of the latter, especially to ursol tartrate. Sodium or potassium bromide enfeebles the blue reaction of diastase with guaiacum and H_2O_2.

The watery or glycerine extract of apples, or the pounded pulp, gives a reaction with ursol tartrate in the absence of hydrogen peroxide, but not potato pulp or its oxidase extract. Living slices of potato give a surface reaction after a day's immersal and the pounded pulp or oxidase extract gives a faint reaction on long standing. According to Moore and Whitley this would be due to its being a "peroxidase" only able to act when peroxidases developed on the surface. If so it is difficult to understand why both apple and potato oxidase and pulp should at once give a blue guaiacum free from peroxide. Rapidly expressed and boiled apple sap contains no trace of hydrogen peroxide and has no perceptible action on ursol tartrate, but if it is added to pounded potato pulp or oxidase extract, the latter now gives a distinct and fairly rapid oxidase action with ursol tartrate, which is not accelerated further by the addition of salt or other sensitisers. Evidently apple sap contains a sensitiser which is absent or deficient in potato pulp and which is not an acid, for no reaction with ursol tartrate is produced by the addition of dilute hydrochloric, oxalic, citric, malic, or tartaric acids to the potato pulp.

Apple ash is rich in potassium, which occurs mainly as phosphate and carbonate. Acid potassium phosphate and normal potassium carbonate have little or no accelerating action on potato oxidase. If they are mixed so as to produce a neutral solution and a small amount added to potato oxidase, the latter will oxidise both ursol tartrate and tannic acid,

on which previously it had little or no action. The readiness with which apple pulp or its extracted oxidase oxidises ursol tartrate and tannic acid is therefore apparently due to the presence of a phosphatic sensitiser. Potato pulp contains phosphates in less amount and in less readily soluble form but if a living slice of potato is immersed in boiled apple sap, a brown layer of oxidised tannic acid slowly forms on the surface where the oxygen and tannic acid come into contact with oxidase and traces of phosphates under optimal conditions for progressive oxidase action. In the living cell the permeability of the cell membrane may determine whether an oxidase and its sensitiser come into contact simultaneously, singly or not at all with an oxidant substance.

The Relation between the Action of Metallic Oxidases and that of an Enzyme.

This question has been fully investigated by H. E. and F. Armstrong, in a long series of papers by themselves and pupils, so far as acids and hydrolysing enzymes are concerned. A few data in regard to certain inorganic oxidases are given beneath, and firstly in regard to the dilution at which a perceptible action is shown. Ferric chloride will give a perceptible blue with guaiacum down to a concentration of 0·0001 per cent., while copper sulphate in the presence of salt gives a faint blue down to 0·00001 per cent. With a 1-per-cent. solution of ursol tartrate in the presence of an equal volume of 1-per-cent. hydrogen peroxide a distinct acceleration of oxidation is shown down to a concentration of 0·0001 per cent. of ferric chloride (0·00003 per cent. in total solution). The oxidation of hydroquinone in the presence of hydrogen peroxide is accelerated by the addition of an equal volume of 0·001-per-cent. ferric chloride but not perceptibly so by lesser dilutions.

One property of an oxidase enzyme is that it may transfer oxygen from one labile compound to another. In the following experiment equal volumes of 1-per-cent. hydroquinone and of ferricyanide of potassium and hydrogen peroxide were mixed, and the time taken to reach a standard shade of brown noted. The top line of the Table gives the concentration of the substance whose amount was varied. The lower rows give the time in minutes required to carry the oxidation to the same stage in each case, and the figures in brackets are the products of the time of reaction multiplied by the concentration of the variant substance. In experiment A, to each 5 c.c. of 1-per-cent. hydroquinone, and of 1-per-cent. hydrogen peroxide, 5 c.c. of potassium ferricyanide of varying concentration were added. In experiment B the hydrogen peroxide was in diminishing concentration; and in C, both the hydrogen peroxide and potassium ferricyanide decreased in concentration correspondingly.

Oxidation of Hydroquinone by Potassium Ferricyanide and Hydrogen Peroxide.

	Concentration of variant substance (per cent.).								
	1.	0·5.	0·25.	0·1.	0·05.	0·01.	0·005.	0·001.	0·0001.
Experiment A ...	17 (17)	20 (10)	23 (5·6)	32 (3·2)	38 (0·76)	48 (0·48)	56 (0·24)	2378 (2·38)	Trace only
Experiment B ...	17 (17)	22 (11)	38 (9·5)	66 (6·6)	Incomplete after 3 days	Faint after 1 week	Trace only		
Experiment C ...	17 (17)	21 (10·5)	32 (8)	1740 (174)	Incomplete after 1 week	Faint after 1 week	Trace only		

The experiments show that in the presence of abundance of hydrogen peroxide an oxidase action is perceptible down to a concentration of 0·001 per cent. of potassium ferricyanide (1 grm. in 100,000 c.c. of water), and that the relative oxidase activity increases with dilution down to a concentration of 0·005 per cent., beyond which it rapidly decreases to nil. the limit being possibly set by extreme conditions of mass action.

Apple Oxidase.

In regard to the influence of KI, KCl, KBr, KF, in all cases the fluoride acted more or less strongly as an antioxidase, while the other salts for the most part exercised a slight retarding influence in the order given, although this was imperceptible with the guaiacum test. The chloride feebly and the bromide more strongly accelerated the decomposition of H_2O_2, and also the oxidation of hydroquinone in the presence of H_2O_2.

When in excess, all four salts strongly retard or even prevent the browning of pounded apple pulp, but without destroying the oxidase. On washing away the excess of the salts and adding H_2O_2, a blue is given with guaiacum and the KBr pulp turns rapidly, the KCl pulp slowly, and the KF pulp very slowly brown. Pulp pounded with 2-per-cent. barium chloride remains colourless, and after three hours, on adding dilute H_2O_2, a feeble evolution of gas is shown and the pulp browns rapidly, but not if previously boiled. If the pulp is washed with water, filtered, and the residue pounded up with a little fresh water, it is able to actively decompose H_2O_2, gives a distinct blue with guaiacum, and on exposure to air slowly browns. Barium chloride, therefore, does not destroy the oxidase but acts as an antagoniser, and

peroxide of hydrogen is able to partially suspend its inhibitory action. In the presence of barium chloride, apple oxidase acts as a "peroxidase," in its absence, as an oxidase.

Strong peroxide of hydrogen destroys the oxidase, and hence pieces of fresh pulp when immersed in a strong solution of pure peroxide remain colourless or show a faint browning along some of the veins. The peroxide, as it penetrates, destroys the oxidase in the protoplasm before it comes into contact with tannic acid. If the pulp is pounded with strong peroxide, the browning is somewhat retarded, but still takes place, since the oxidase, tannic acid, and peroxide are in contact simultaneously and react before the oxidase is destroyed. Dilute peroxide accelerates the browning of pounded pulp.

Lead acetate appears at first sight to be incapable of preventing the browning of pounded apple pulp. The pulp darkens to yellowish or greenish brown, and even after 24 hours retains a distinct but enfeebled power of decomposing hydrogen peroxide and turning guaiacum blue. The darkening is, however, partly due to the precipitation of yellowish-white lead tannate and the retention of the power of turning blue is easily explained, for lead acetate itself gives a strong blue with guaiacum in the presence of hydrogen peroxide, and can therefore act as an oxidase. Any poison which destroys the oxidase also removes the power of turning brown, and the pulp of apples turns brown when soaked in bulk in metallic poisons, because the slowness of penetration allows the cell to be killed and browning to occur before the oxidase is destroyed. Barium chloride, however, inhibits oxidation without destroying the oxidase ferment.

The Chromogen of the Apple.

In the apple the chromogen is known to be a form of tannic acid. In a previous paper it was shown that tannic acid vacuoles appeared in the protoplasm of pulp cells immersed in methyl blue or ferric chloride, so that the assumption that the whole of the tannic acid was present in the cell sap did not appear to be correct. No such vacuoles could, however, be detected in living pulp cells, or in the protoplasm of cells killed by heat prior to staining. Further, although pulp from which most of the sap has been expressed turns, if anything, darker with $FeCl_3$ than before, this may be merely because the tissue is more compacted. In addition, if slices of pulp are subjected to very strong pressure between wads of filter paper until the pulp is quite dry, on moistening with water the pulp remains colourless or the veins may turn brown, and no distinct tannic acid reactions are given with $FeCl_3$, KCN, or iodine and ammonia. Microscopic examination shows

that the protoplasm is still present in the pulp cells, and very careful testing shows that a trace of tannic acid may adhere to it. The colourless pulp from which the sap has been removed, after pounding with water, gives distinct oxidase reactions. Even after repeated extractions of pounded pulp with absolute alcohol, a little tannic acid adheres to the pulp, which turns green and then brown with ferric chloride, but remains colourless in air owing to the destruction of the oxidase by the alcohol. Apparently, therefore, the tannin vacuoles which may appear in the protoplasm are factative and are due to the methyl blue or ferric chloride meeting tannic acid in the dying protoplasm. They are possibly analogous in origin to the vacuolation which may be produced in dying protoplasm by various chemical agencies. Using concentrated solutions of methyl blue or ferric chloride they do not appear in the surface cells but only in those at a certain depth. Evidently it is a condition for their formation that the cell should die slowly.

In regard to the form of tannic acid present, this is not gallotannic acid, but is more closely allied to mangrove tannin. Thus with calcium hydrate it gives white turning red and not blue. As it gives green with ferric chloride instead of blue or black, it is presumably a catechol yielding tannin and not a pyrogallol tannin. The green given by the expressed sap or pounded pulp with ferric chloride rapidly changes to brown, especially if the material is nearly neutralised. Pounded pulp, allowed to brown, darkens to almost black with $FeCl_3$, and shows no green colour at first, but this does not indicate a production of gallotannic acid by oxidation, but may be due to the superposition of the two colours.

If a dilute solution of gallotannic acid (0·05–0·1 per cent.) is divided into three parts, A, B, C, and C is saturated with common salt, on adding an excess of 10-per-cent. ferric chloride to B and C a green liquid is formed, whereas with a drop only of ferric chloride A gives a blue-black liquid. On boiling, A forms a blue-black precipitate, B forms a brown liquid, and C forms a green or yellowish-green liquid. The blue-black colour reaction of gallotannic acid with ferric chloride is therefore capable of various modifications.

Gallotannic acid gives a blue-black precipitate with ferric chloride in the presence of oxalic acid. If ferric chloride is added to a slight excess of gallotannic acid and apple sap added to the blue-black liquid, it turns green, just as the sap does with $FeCl_3$, but on standing the first liquid takes a bluish tinge, while the second becomes brown. Hence, apparently, the different reactions are partly due to differences between gallotannic acid and the tannic acid of apple pulp and partly to the substances which accompany the latter, but are not entirely due to the presence of free organic acid in the

sap. There is nevertheless a close correspondence between the behaviour of gallotannic acid and of the apple greening tannin to metallic oxidases.

Thus dilute gallotannic acid solutions give a turbid liquid with copper sulphate, but in the presence of salt a clear liquid. After one day a pale brown precipitate separates out from both liquids. With peroxide of hydrogen gallotannic acid is unaffected, but in the presence of copper sulphate, sodium chloride, and hydroxyl the gallotannic acid immediately develops a strong brown colour.

Clear boiled apple sap shows practically identical reactions. Colourless pulp pounded with copper sulphate remains unchanged, with copper sulphate and salt it develops a faint, barely perceptible, brownish tinge in the parts exposed to air, and with copper sulphate and hydroxyl it develops a pale brownish tinge, which is immediately intensified on adding salt. Pounded up with copper sulphate, salt, and hydroxyl, boiled apple pulp is browned as deeply or almost as deeply as by the full action of the natural oxidase, whereas potato pulp is unaffected.

The Chromogen of the Potato.

The chromogen of the potato appears to be not a tannin compound, but some substance related to tyrosin. Freshly pulped potatoes acquire a purplish-brown tinge in air, removed by washing and squeezing and returning to a less and less extent with each washing on standing. The chromogen appears to be dissolved in the cell sap and the product of oxidation to be soluble in water. In the apple when the tannin is oxidised inside the cell, it is rapidly absorbed and permanently retained by the protoplasm.

No distinct traces of tannic acid can be detected in resting potatoes by ferric chloride or other tests. In 10-per-cent. sodium hydrate, potato pulp becomes transparent but remains practically colourless.

If tyrosine is added to fresh potato pulp, or to a diluted glycerine extract, a purplish-brown colour is given more rapidly and deeply. This colour is also soluble in water, and hence presumably the chromogen is tyrosin.

Boiled potato pulp or sap remains colourless. Cubes of pulp treated with absolute alcohol remain colourless both in air and in water. The alcoholic extract is pale yellow, and has no oxidase properties. If evaporated *in vacuo* and water and a glycerine extract of oxidase added no change of colour occurs. Treatment with absolute alcohol seems, therefore, to either destroy or precipitate both oxidase and chromogen. If the pulp from alcoholic extraction is pounded with water and a glycerine extract of oxidase added the pulp remains almost colourless, but the supernatant liquid turns

purplish-brown. Hence the chromogen after precipitation by absolute alcohol can be dissolved again in water. The expressed sap from potatoes turns black in a day. If boiled, treated with HCl or absolute alcohol a dirty white or grey precipitate is thrown down, and in the case of the absolute alcohol the supernatant liquid is colourless and devoid of both chromogen and oxidase.

Potato Oxidase.

Potassium chloride and bromide feebly and potassium fluoride strongly retard its oxidase action on ursol tartrate and H_2O_2, on hydroquinone and H_2O_2, and on pyrogallol and tyrosin. The addition of 5-per-cent. lead acetate to the glycerine extract throws down a bulky white precipitate which gives no blue with guaiacum, but shows an active power of decomposing H_2O_2, and in the presence of the latter gives a distinct blue with guaiacum. Lead acetate itself, however, gives a peroxide reaction with guaiacum. Potato oxidase by itself is unable to oxidise tannic acid even in the presence of H_2O_2, but if a little neutral solution of sodium phosphate is added, the liquid turns slowly pale brown in the absence of H_2O_2, and more rapidly a darker brown in its presence.

Potato oxidase is peculiar in the readiness with which it oxidises tyrosin, which most metallic oxidases only oxidise slowly and usually only in the presence of H_2O_2. Possibly this peculiarity is due to the presence of a specific sensitiser in the potato. Potassium phosphate appears to act as a feeble sensitiser to the oxidase action of potassium ferricyanide on tyrosin, but the nature of the sensitiser in the potato is doubtful. That such may be present is indicated by the fact that potato extracts may sometimes be obtained apparently capriciously, which while still reacting strongly to guaiacum only react feebly to tyrosin and conversely. This is particularly the case when partially sprouted tubers are used and fractional extractions made.

The Extraction of the Oxidase.

The oxidase of the potato is either more active or more abundant than that of the apple, whereas the chromogen of the apple is more abundant than that of the potato. Absolute alcohol not only does not extract the oxidase but destroys it, but if the pulp of the apple or the potato is pounded with pure glycerine and filtered, the filtrate shows strong oxidase properties. Glycerine also extracts some of the chromogen in each case, and hence if diluted with water and exposed to air it darkens rapidly. If the glycerine extract is concentrated by soaking cubes of material in glycerine, and then pounding up with a little fresh glycerine, it is obtained as a clear yellowish

liquid which may remain colourless and may show a strong power of decomposing H_2O_2 and turning guaiacum blue for a month or more at 12–14° C. The glycerine extract of the apple if half diluted with water turns brown, and shows faint oxidase reactions after 10 days and none after 15 days. The process of browning may weaken the oxidase in the same way that it is weakened, and finally destroyed, by decomposing an excess of hydrogen peroxide.

The addition of a little of the glycerine extract to the sap from potato cubes just killed by heat causes the production of a purplish-brown colour, whereas the same amount of extract added to pure water produces no distinct change of colour.

When pulp is pounded with glycerine filtering is difficult and prolonged. The best mode of obtaining the oxidase extract is by cutting the pulp into minute cubes or thin slices, and placing these in glycerine for five minutes, then pouring off the now diluted glycerine and replacing by fresh glycerine. In this they may soak for 1–2 days. The glycerine can then be strained off, and, if not diluted with water, keeps without discolouring.

If potato pulp is left in contact with glycerine it discolours on the exposed surface in a few days, but, if well covered by glycerine, remains uncoloured. Pounded pulp repeatedly extracted with an excess of glycerine for three weeks and thoroughly washed with water remains colourless on exposure to air, after the addition of fresh glycerine extract. Hence the glycerine and water can extract the whole of the chromogen from the pulp.

Nevertheless, the oxidase appears to cling with some tenacity to proteids of the cell. Thus pounded potato pulp was allowed to brown, and then washed till quite colourless, and the washing continued for an hour. On testing the pulp it still decomposed hydrogen peroxide energetically and gave oxidase reactions, while another portion remained colourless after the addition of fresh glycerine extract. Evidently the chromogen is easily removed by washing, but not the oxidase. Nevertheless, the latter is soluble in water, for the clear filtrate from pulp pounded with water shows distinct, though not very strong, oxidase reactions.

The glycerine extract of apple pulp shows feebler oxidase properties than that of the potato, and, when diluted, slowly turns reddish-brown on exposure to air.

A simple mode of rapidly obtaining an extract of potato oxidase free, or nearly free, from the chromogen is to pound up to a paste, wash with water, remove the pulp with a strainer, leaving the starch behind. Squeeze out the excess of water, pound up with fresh water, settle and

decant and filter the clear liquid. In this way a strong watery extract can be obtained suitable for immediate use, and practically free from the chromogen. The oxidases of all the plants examined appear to be soluble in water, or, at least, to pass through a filter paper. By half saturation with alcohol they can be precipitated, possibly clinging to precipitated proteids, but with excess of alcohol are attenuated and finally destroyed.

The Distribution of the Oxidase in the Cell.

Rapidly expressed and filtered apple sap does not decompose hydrogen peroxide, and gives no blue with guaiacum. Later filtrates, when the odd pulp cells on the filter paper become brownish, show a very feeble decomposition of H_2O_2, and give a faint blue with guaiacum on long standing.

If fragments of fresh pulp crushed between filter paper till dry are added to colourless apple sap, the latter only becomes brownish-yellow after three days, and the former browns distinctly, whereas in water it remains practically colourless.

Apparently, therefore, the oxidase is in the protoplasm, and not in the cell; sap and the browning is more readily produced when the tannic acid is inside the cell than when it is outside.

In the case of the potato the expressed sap, however obtained, rapidly discolours in air, and contains both oxidase and chromogen. If, however, slices of fresh potato are immersed in colourless apple sap (filtered and boiled), they slowly turn deep brown. The brown colour is on the surface layers, and is mainly in the protoplasm, which darkens strongly, and the cell-walls slightly, on adding ferric chloride. The tannic acid of the apple sap and the oxidase of the potato meet mainly in the protoplasm of the latter, and the sap outside is only slightly discoloured, and, if plenty of potato is used, contains much less tannic acid. Potato oxidase will therefore oxidise apple tannin, but much more slowly than apple oxidase *in situ*, and it appears to be located in the protoplasm of the potato cells. Possibly the potato oxidase may be aided by the less soluble phosphates retained by the potato cells, or may work better in a less acid medium.* At least, if the apple sap is nearly neutralised by the addition of dilute ammonia, the browning of the potato slices in apple sap is slightly accelerated, and these become very dark or black on the addition of ferric chloride. On the other hand, if an excess of apple sap is used, the browned potato pulp loses its oxidase properties, and, as the liquid gains none, the oxidase has evidently been destroyed.

* According to Hunger ('Bull. d. D. Bot. Gesell.,' vol. 19, p. 374 (1901)), tannins and glucose often mask the presence of an oxidase or prevent its action. This certainly does not apply in the case of the apple.

Colour and Oxidase Action.

Owing to their striking character most attention has been directed to those oxidase reactions which are accompanied by a production of colour. The parsnip and carrot have fairly strong oxidases in their cortex, phloem and cambium, but the former has no chromogen, the latter none oxidising further on death. The most important oxidase reactions are in fact probably those unaccompanied by any colour change, and in some cases coloured bodies may be rendered colourless by oxidase action.

Thus a living slice of potato stained with a watery solution of gentian violet becomes slowly paler when kept moist in air. The extracted oxidase of potato slowly partially decolorises gentian violet, and the action is hastened by the addition of small quantities of H_2O_2. Gentian violet may be heated with H_2O_2 without being decolorised but if a drop of a mixture of $CuSO_4$ and sodium or potassium chloride is added to the hot liquid, the latter rapidly becomes colourless.

Copper sulphate alone is much less effective. Similar results are given by eosin, indigo carmine* and methyl blue (pale purplish) but at temperatures below 50° C., which is the highest to which organic oxidases can be raised with safety, the reductions by the metallic oxidase are very much slower than at 95° C. to 100° C. Further, in the case of organic oxidases, dilute solutions must be used so as to avoid poisoning the oxidase. Even then only partial decolorisation is shown and this is often difficult to distinguish from effects due to absorption. Using a strongly oxidase diastase a faint decolorisation was shown in the presence of hydrogen peroxide with dilute solutions of methyl blue and eosin but none with gentian violet or indigo blue with or without peroxide of hydrogen.

Since copper sulphate and salt can decolorise indigo carmine, an oxidase can also act as a reducing agent in the presence of an excess of hydrogen peroxide, particularly at high temperatures.

The Destruction of Oxidase by Heat.

Although oxidases are not necessarily proteins, cell oxidases seem to adhere closely to proteins, and it is possible that it is the coagulation of the latter by heat that renders the oxidases inactive. Their destruction by absolute alcohol might arise in the same way.

The glycerine extracts of both apple and potato develop coagulated particles on boiling, at the same time that they lose their oxidase properties.

* Prolonged boiling with excess of H_2O_2 partially reduces indigo carmine to a brown or greenish-brown liquid.

If a dilute solution of copper sulphate and salt is mixed thoroughly with an excess of egg albumin, a greenish-white precipitate is formed, but the liquid will still give a strong blue with guaiacum. After boiling, the filtrate gives no blue with guaiacum, and the coagulum gives a greenish colour only. Boiling with coagulable proteid would apparently in this case practically destroy the "oxidase" reactions of copper sulphate and salt, and would entirely remove the oxidase from its solution in water.

Where, however, a cell oxidase is a metallic salt not combined with or associated with proteins, the oxidase properties may be retained after boiling. Instances of these are already known,* and where boiling removes the direct oxidase action of an extract but this returns slowly after cooling, this may be due to the conversion of a "per" salt into a "proto" salt or *vice versâ*. Further, a solution of potassium ferrocyanide on standing in light develops traces of ferricyanide and then becomes able to give a direct blue with guaiacum.

The Resistance of Oxidases to Drying and Keeping.

According to Moore and Whitley (*l.c.*), apple and carrot gratings dried at 45° C. for eight and three days respectively lost all their oxidase. Potato pulp was ground up, the excess moisture pressed off and the pulp spread in thin layers to dry in air at 15° C. This formed a grey powder when ground. It decomposed H_2O_2 moderately actively, mixed with water gave a very faint blue with guaiacum, with ursol tartrate and H_2O_2 the liquid darkened slowly to brown and ultimately purplish. Drying makes the oxidase cling firmly to the proteids of the pulp. These properties were shown by the powder even when three months old. Similarly mere drying did not destroy the oxidase in apple pulp from which the sap had been pressed out before pounding and drying, and the properties were retained uninjured for over three weeks in the dry condition. Moore and Whitley's results may have been due to the non-removal of the sap or to the higher temperature used.

Even in solution oxidases may retain their properties for a long time. The glycerine extract of potato oxidase, covered, but in contact with air, darkened slowly at 12–15° C., gave after two months a distinct reaction with guaiacum, a slow change through brown to purple with guaiacum, a slow change through brown with ursol tartrate, and a moderately active decomposition of H_2O_2. At three months it gave no blue with guaiacum alone, a faint blue with guaiacum and salt, stronger with guaiacum and H_2O_2, and very slow browning with salt and ursol tartrate, stronger with H_2O_2. At four months it gave a faint blue with guaiacum and H_2O_2 and a moderately active decomposition of

* See Lafar, 'Technische Mycologie,' vol. 1, p. 675 (1907).

H_2O_2, but no other oxidase reaction. At five months it produced a weak decomposition of H_2O_2 but no other oxidase reaction. At the eighth month the decomposition of H_2O_2 became practically imperceptible. In this case, by gradual attenuation, the same oxidase became first a "peroxidase" and finally a pure "katalase."

Paraphenylenediamine Test for Oxidase.

As is well known, this substance forms an exceedingly sensitive test for oxidases, and goes through a remarkable series of colour changes under their action. The full series of colour changes is green, then blue, then brown, then violet, darkening, and in strong solutions forming a black precipitate, but according to circumstances, or if the oxidase action is very intense or very feeble, one or more of these changes may be omitted or modified. The chief objections to the reagent are the readiness with which decomposition or oxidation takes place naturally and its excessive sensitivity. Gruss* recommends the use of the tartrate of paraphenylenediamine (ursol tartrate). This dissolves readily in water, and a pinch of the dry salt can be dissolved in water for each test. Any colour change in the clear solution is readily perceptible, and there is no alcohol present to interfere with the reaction. Further the dry tartrate keeps indefinitely. It is, however, not so sensitive and responds more slowly. On the other hand, it will often give a full colour series, where the alcoholic solution of paraphenylenediamine gives a single colour change only, which, when slow, may be confused with its natural slow darkening on exposure to air.

Neither alcoholic paraphenylenediamine nor the tartrate respond to all oxidising agents. Thus nitric acid appears if anything to exercise a reducing rather than an oxidising action. It does not produce any colour sequence, and if a little dilute nitric acid is added to potato pulp turned green or blue by ursol tartrate and peroxide of hydrogen, the pulp immediately becomes pale in colour.

The reactions with those metallic salts capable of turning guaiacum blue are of interest.

Silver nitrate forms a grey precipitate, slowly darkening, with alcoholic paraphenylenediamine, but in the presence of hydrogen peroxide the colour sequence, green, brown, ruby, violet is given, and the same is given with silver nitrate and ursol tartrate, whereas in the presence of hydrogen peroxide the change is from green to brown only. Ferric chloride gives the full colour sequence (green, brown, violet, or purple) with alcoholic paraphenylenediamine,

* 'Biologie der Enzyme,' 1912.

but in the presence of hydrogen peroxide gives a reddish-brown at once. With the watery solution of the tartrate the colour sequence is also given. The presence of free nitric, sulphuric, hydrochloric, citric, or tartaric acids prevents or delays the production of an oxidation colour sequence with ferric chloride, but this takes place readily in the presence of 10 per cent. oxalic acid. With soluble copper salts (sulphate, acetate, chloride), alcoholic paraphenylenediamine darkens directly without showing any colour sequence, but if the solutions are dilute, and salt and hydrogen peroxide are present, a partial colour sequence from brown to violet or purple is shown.

With the watery solution of ursol tartrate no colour change is given with copper acetate, sulphate, or chloride in the presence or absence of sodium chloride. With the sulphate and acetate an apparent colour sequence of green to brown is given on the addition of hydrogen peroxide, but this is partly due to the fact that the peroxide gives a greenish colour with copper, and ursol tartrate slowly browns in the presence of hydrogen peroxide. With copper chloride, however, a violet or purple tinge ultimately appears, and a full colour sequence (green, brown, violet, or purple) is given with copper sulphate and copper acetate in the presence of salt and hydrogen peroxide. If, however, the solutions are very dilute the colour change is slow, and is direct to brown.

Gruss* suggests that the direct oxidation to brown is due to molecular oxygen, and that the colour sequence is the result of the action of atomic oxygen. The data given above, however, yield no support to this view. The colours produced seem to depend to some extent upon the relative degrees of dilution and intensity of action. Colour may be intramolecular or extramolecular, *i.e.* due to the absorption or modification of light rays at the surfaces of molecules or of molecular aggregates. In the latter case if the peculiar aggregation is broken up when the material is in solution the colour may disappear or be modified. It is quite possible that the colour sequence with paraphenylenediamine is the result of temporary molecular aggregations during the process of oxidation which react differently to light rays, and whose production depends more upon relative mass action than upon any other factor, this determining molecular aggregations of material in various stages of oxidation.

Ursol Tartrate Test for Lignin.

This delicate and striking reaction is best shown with boiled or dead tissues by placing them in the watery solution. It is a reaction comparable with the phloroglucin test, and is shown in the absence of free oxygen, acid,

* *Loc. cit.*, p. 11.

or light. The colour given is brown or brownish red. It picks out the wood vessels in a slice of boiled potato or carrot in brownish red without affecting the other tissues. The bundles or vascular network on the inner surface of orange or lemon peel are coloured bright red on a white ground, looking like blood vessels injected with carmine. Conifer wood or a match also colours bright red. As a direct test, it is simpler to apply than any other lignin test, and the colour is confined to the walls of the vessels or tracheides. In testing tissues for oxidase this reaction must be borne in mind.

The Action of Paraphenylenediamine on the Oxidases of Apple Sap and Potato.

Apple pulp turns violet, in a few minutes rapidly darkening to blue, with alcoholic paraphenylenediamine in the absence as well as in the presence of peroxide of hydrogen. In the former case the blue colour remains permanent for an indefinite length of time, whereas in the presence of peroxide of hydrogen the oxidation is ultimately completed to a dark brown or black.

Potato pulp remains colourless with alcoholic paraphenylenediamine for one or more hours, but on long standing the liquid acquires a brown colour, tinged with violet, and the pulp a weak but distinct violet tinge. In the presence of peroxide of hydrogen a violet colour is rapidly produced, but changes to brown in the presence of an excess of peroxide of hydrogen.

Using a watery solution of ursol tartrate, apple pulp develops a violet or blue colour in the absence and presence of peroxide of hydrogen, appearing first in the veins and persisting for a long time. With potato pulp and in the presence of peroxide of hydrogen a green colour is shown passing through blue rapidly to a slate colour. In the absence of peroxide of hydrogen potato pulp remains colourless, gradually acquiring a slight brown colour in two days, but with no signs of any colour sequence, and the brown is hardly stronger than that produced in pieces of boiled egg albumin used as a control. In all cases no colour sequences were produced by boiled apple or potato pulp. In needing hydrogen peroxide to produce a colour sequence with ursol tartrate, potato oxidase therefore resembles copper sulphate and salt, and, similarly, both the vegetable oxidase and the metallic oxidase give a blue colour with guaiacum in the absence of hydrogen peroxide. That is they are oxidases to guaiacum, "peroxidases" to ursol tartrate.

On the other hand, apple oxidase resembles ferric chloride in its ability to produce oxidase colour changes with both ursol tartrate and guaiacum in the absence of hydrogen peroxide.

The Potassium Iodide and Starch Test for Oxidase in Living Tissues.

Moore and Whitley consider that where a plant extract gives a blue with guaiacum without the addition of hydrogen peroxide being necessary, this is due to the production of peroxides by the dying protoplasm during extraction or to their presence in the guaiacum solution. If H_2O_2 is added to a solution of potassium iodide, iodine is liberated and gives the usual blue with starch. On applying potassium iodide to the freshly cut surface of a potato a blue is also slowly formed, as well as with the cut surface of an apple or carrot smeared with starch. Bach and Chodat* consider this to prove that the living cells develop peroxides. If, however, the material is pounded to a fine pulp and potassium iodide applied to the surface no liberation of iodine takes place, and yet in freshly pounded pulp any peroxides produced by drying cells should be more abundant than in a freshly cut surface. Further, the pounded pulp gives a strong blue with guaiacum without the addition of hydrogen peroxide being necessary. With strong potassium iodide, pounded pulp browns, and the starch grains swell but remain uncoloured, although staining readily when free iodine or when hydrogen peroxide is added. As a matter of fact the liberation of iodine from potassium iodide appears to be due to the oxidase present in the tissues used. If a slice is boiled and a fresh surface cut no liberation of iodine is shown. Actual tests showed that slices soaked in hydrogen peroxide contained some of the latter undecomposed after again boiling.

Certain metallic oxidases such as ferric chloride, black oxide of manganese and potassium ferricyanide will also liberate iodine in a solution of potassium iodide. Hydriodic acid is a substance which readily undergoes oxidation with a production of free iodine, and dilute hydrochloric acid liberates free iodine at the surface of a solution of potassium iodide, giving a blue colour in the presence of starch. Bach and Chodat† have shown that the oxidases in the sap of plants can decompose hydriodic acid, although Aso‡ considers this action to be due to the presence of nitrates or nitric acid. The solution of potassium iodide we may suppose to contain in addition to ions and undissociated molecules of KI also KHO and HI. The latter would be liable to oxidation by organic oxidases when applied on one side of a semipermeable membrane. The action is naturally favoured by the presence of free acid, and is only shown by tissues rich in oxidase. The apple, potato and carrot, which are all acid, give the change readily and the iodine is liberated first over the parts rich in

* 'Ber. d. D. Chem. Gesell.,' vol. 35, p. 2464 and p. 3943 (1902).
† 'Ber. d. D. Chem. Gesell.,' vol. 37, p. 36 (1904).
‡ 'Bull. Coll. Agric.,' Tokio, vol. 5, p. 481 (1903).

oxidase, such as the phloem and cortex of the carrot, the veins of the apple and potato. A potato kept until the tuber was watery but still acid, and in which the oxidase had nearly disappeared, showed no power of liberating iodine from potassium iodide.

The cut surface of the parsnip is neutral or feebly alkaline and, although rich in oxidase, a cut surface shows only a feeble power of liberating iodine from potassium iodide over the phloem ring and outer cortex after many hours' exposure to air. The acid pulp of the orange and lemon, which contains no oxidases, is unable to produce any liberation of iodine, nor does the wood cylinder of the carrot, which is usually more acid than the cortex but contains hardly any oxidase.

This action is evidently due to the oxidase and not to the free acid. The extracted oxidase, however, like pounded pulp is unable to produce any liberation of free iodine from potassium when tested in the usual way. Possibly this is because any iodine liberated would at once attack and destroy the plant oxidase where this was in immediate contact with potassium iodide. Free iodine does actually destroy potato oxidase. Hence to produce any progressive liberation of iodine sufficient to stain the starch the oxidase and potassium iodide would need to be separated by a semi-permeable or colloidal membrane, such as is formed by the cell walls on the cut surface.

If pounded potato pulp or filter paper pulp saturated with a glycerine extract of oxidase is covered by a layer of gelatine containing starch or of starch paste, and a little potassium iodide poured on top when the colloid layer has set, after one day a more or less prominent violet line appears on or close to the pulp. Apparently the oxidase is only able to liberate iodine from potassium iodide when the latter diffuses slowly to it, and this is possibly a question of relative mass action and osmotic separation.

In any case the liberation of iodine from potassium iodide on the cut surface of a living tissue can be used as a confirmatory test for the presence of an oxidase. It does not indicate the presence of hydrogen peroxide or of any special "iodoxidase."

The Influence of Anæsthetics on Oxidase Action.

Ether.—Small cubes of potato soaked in saturated ether water for a day and then exposed to air darkened distinctly. Pulp triturated with ether darkened slightly, and gave strong oxidase reactions and decomposed H_2O_2. The clear ether extract had no oxidase properties. Apple pulp pounded with excess of ether turns a deep brown, but a little more slowly than in the absence of ether. The ether extract is yellow, not owing to tannin but

to etiolin. The pulp gives strong oxidase reactions, but only decomposes H_2O_2 feebly or not at all. If allowed to dry in the air the oxidase reactions are feebler, but the decomposition of H_2O_2 a little more active. If the potato pulp ground up with ether is left in contact with it and tested at hourly intervals, it ceases to give distinct oxidase reactions in the following order:—Ursol tartrate and H_2O_2, guaiacum, ursol and H_2O_2, guaiacum and H_2O_2, decomposition of H_2O_2. Hence a substance which is at first a "peroxidase," an oxidase and a "katalase," as it is attenuated, becomes a "peroxidase" and "katalase," and finally a "katalase" only.

Chloroform.—Apple pulp pounded with an excess of chloroform turns a yellowish-brown, deepening slowly on exposure to air. The pulp does not decompose H_2O_2; it gives feeble or doubtful oxidase reactions, which in the case of guaiacum are rendered more distinct by the addition of H_2O_2, but not in those of ursol or its tartrate. If the chloroformed pulp is dried in air and powdered up, it regains a weak power of decomposing H_2O_2, and shows stronger but still feeble oxidase reactions. Potato pulp triturated thoroughly with excess of chloroform, after the latter had been allowed to evaporate, gave no oxidase reactions with ursol or with the tartrate and H_2O_2, a pale blue with guaiacum on standing, given at once in the presence of H_2O_2, and produced a very feeble decomposition of H_2O_2. The chloroform apparently attenuates or retards oxidase action much more than ether does.

Neither chloroform nor ether inhibits the action of metallic oxidases such as copper sulphate and salt, ferric chloride, black oxide of manganese, potassium permanganate, or potassium ferricyanide, but in certain cases chloroform retards or inhibits the decomposition of hydrogen peroxide. Thus if a mixture of copper sulphate and salt is shaken up with an excess of chloroform a temporary precipitation film like an exaggerated surface tension film forms on the surface of the chloroform, and on adding H_2O_2 an occasional large bubble may form beneath this film, lifting it up like a skin, but in the liquid above no decomposition of the H_2O_2 takes place. If, however, the chloroform is removed by evaporation or the liquid warmed to start the decomposition it continues indefinitely. Chloroform itself does not decompose H_2O_2, and saturation with ether slightly lessens the decomposition without arresting it. Similar results were given with ferric chloride, except that the action of the ether is stronger, and if the ether or chloroform is removed by boiling the liquid becomes reddish-brown and loses the power of decomposing H_2O_2, whereas if removed by evaporation at a low temperature the power of decomposing fresh hydrogen peroxide is regained. Chloroform added to potassium ferrocyanide and H_2O_2 merely changes a rapid stream of small bubbles into a slow stream of occasional larger

bubbles, and still less influence was exercised upon the decomposition produced by potassium permanganate and black oxide of manganese, although in the latter case some remarkable surface tension effects were exercised.

Hydrogen peroxide is readily soluble in ether, which will in fact remove it from a watery solution. It is only sparingly soluble in, chloroform, for, although the chloroform solution will not give any blue with chromic acid, it gives a feeble reaction with a watery solution of starch and potassium iodide or with ferrous sulphate. Chloroform prevents the ether-chromic acid reaction for hydrogen peroxide being given but not by destroying the hydrogen peroxide. In fact the hydrogen peroxide can be shaken with chloroform and the latter then boiled off without the former being destroyed. The retarding action on peroxide decomposition produced by ether might depend upon whether the "katalase" salt dissolves in it as well as in water, since otherwise the hydrogen peroxide might be removed from katalase action except at the contact surface. In the case of chloroform any bubbles produced form mainly below the surface tension film, although both hydrogen peroxide and the katalase salt may be present in abundance in the liquid above. The chloroform apparently acts as an "anæsthetic" to "katalase" chemical action.

The Oxidases of the Lemon and Orange.

According to Moore and Whitley there are no "peroxidases" in the pulp or rind of these fruits. This is hardly the case, as no allowance was made for the effect of the acid in the pulp or of the oils in the skin. Quarters of the pulp were squeezed dry in a press between blotting paper, and collected until a sufficiency of clean material free from acid was obtained. This gave no reaction with guaiacum alone and none with ursol tartrate except that the fragments of the tracheæ coloured brownish-red. On adding a drop of peroxide of hydrogen a pale but distinct blue was given with guaiacum and a slow change to violet with ursol tartrate. The pounded pulp does not decompose hydrogen peroxide appreciably. On dissecting out the vascular bundles and applying ursol tartrate and peroxide of hydrogen, all the veins right down to the stalks of the endocarpal hairs turned green, then brown, then violet, but no other parts. They also showed a feeble power of decomposing H_2O_2. After soaking in orange or lemon juice for some hours or after boiling no oxidase reaction was given but the walls of the tracheæ gave a bright red lignin reaction, making the bundles look like blood-vessels injected with carmine.

The Oxidase of the Carrot.

According to Moore and Whitley the "peroxidase" of the carrot is most abundant in the protoxylem. They were either misled by the lignin reaction or mistook the central wood cylinder of the carrot for pith. With both guaiacum and ursol tartrate in the presence of H_2O_2 the oxidase reaction is given first in the cambium, cambium segments and phloem. The central wood cylinder is the last part to show any true oxidase reaction. All parts decompose H_2O_2, and in ursol tartrate alone a green colour slowly appears along the line of the cambium, while the vessels in the wood within colour reddish-brown. The latter colour appears in a boiled section but not the former. We are evidently dealing with a somewhat weak oxidase, most abundant in the cambium and phloem, next in the outer cortex and least of all in the central wood cylinder.

According to Moore and Whitley the cut surface of a carrot rapidly develops peroxides and will then give a blue with guaiacum without any addition of H_2O_2. It is difficult to see how this explanation can apply to a tissue like that of a carrot which rapidly decomposes peroxides, or at least peroxide of hydrogen, or to a section of carrot immersed in a large quantity of a watery solution of ursol tartrate, in which the reaction is slowly given by the uncut cells beneath the surface, and where any peroxides formed in the uninjured cells would be washed away. Actual tests failed to detect any peroxide of hydrogen in living or dead carrot tissue.

The Oxidase of Red Beetroot.

In spite of its red colour the expressed sap of the beetroot shows a strong reaction with guaiacum, but is difficult to use with other oxidants. Hence the sap was squeezed out, the pulp washed, the excess of water squeezed out, and the residue pounded with glycerine, the first portion of which was thrown away. In this way a pale pink strongly active oxidase was obtained, which closely resembled potato oxidase. It reacted to guaiacum and tyrosin in the absence, but to ursol tartrate only in the presence, of hydrogen peroxide. It has no action on tannic acid by itself and only a feeble one in the presence of sodium phosphate. It has a weaker power of decomposing hydrogen peroxide than potato oxidase and the peroxide appears to inhibit its action on pyrogallol, but acts as a sensitiser in the case of ursol tartrate.

The pounded pulp reacts strongly and rapidly to ursol tartrate in the presence of hydrogen peroxide but only slowly and faintly or not at all in its absence. Neither the pulp nor the expressed sap appears to contain any perceptible amount of chromogen capable of oxidation.

According to Bertrand* the sugar-beet contains an oxidase capable of oxidising tyrosin which he terms "tyrosinase," and this, according to Gonnermann,† oxidases tyrosin to homogentisinic acid, which darkens rapidly by direct oxidation to red, brown, or black. In the red beet the amount of tyrosin present appears to be too small to appreciably affect the neutral red colour. It may undergo oxidation, while the plant is living, and hence be unable to accumulate.

The Oxidase of the Parsnip.

The parsnip differs from all the other vegetables used, in that a cut surface is neutral or faintly alkaline instead of acid, and it resembles the carrot in containing no chromogen oxidising on death. Neither carrot nor parsnip oxidase will directly brown boiled potato or apple pulp, but if a little sodium phosphate and H_2O_2 is added, they will cause tannic acid, apple pulp and apple juice to brown distinctly and with fair rapidity.

In both carrot and parsnip the oxidase is mainly present in the phloem and outer cortex, and that of the carrot appears to be a little more abundant. Hence of similarly prepared watery or glycerine extracts the former is a little more active than the latter.

	Beetroot oxidase.	Carrot oxidase.	Parsnip oxidase.
Guaiacum	+ + +	+ +	+ +
Do. + H_2O_2	+ + +	+ + +	+ + +
Ursol tartrate			
Do. + H_2O_2	+	+	+
Pyrogallol	+ +	+	
Do. + H_2O_2	+	+ + +	+ + +
Hydroquinone			
Do. + H_2O_2	+	+	+
Gallic acid			
Do. + $_2H_2O$	+	+	+ + +
Tannic acid			
Do. + H_2O_2			
Tannic acid and sodium phosphate			
Do. + H_2O_2		+	+
Tyrosin	+ +		
Do. + H_2O_2	+		

Both ursol tartrate and hydroquinone when applied to a cut surface of a carrot or parsnip show signs of oxidation particularly over the phloem ring. This is probably because the oxygen is more concentrated at the surface and also a greater mass action is exercised upon the inwardly diffusing oxidant. No assumption of the production of peroxides in the tissue is necessary to explain the action. The addition of magnesium sulphate or of potassium

* 'Bertrand, 'Compt. Rend.,' vol. 122, p. 1215.

† Gonnermann, 'Pflüger's Archiv,' vol. 82, p. 289 (1900).

phosphate to carrot or parsnip oxidase causes it to give a faint trace of browning in one day with tannic acid which is not increased by the addition of hydrogen peroxide. None of the other salts which could be made up from the ash constituents (excluding iron salts) exerted any sensitising oxidase action. The oxidase of the beetroot and potato appear to belong to one class (betase, potatase, dahliase, russulase), those of the carrot and parsnip to another, while the chief peculiarity of apple oxidase, namely, the readiness with which it oxidises tannic acid, appears to be due to the presence of a sensitiser such as potassium phosphate. Apple oxidase appears to have some resemblance to a weak form of laccase which is also able to oxidise tannic acid.

Summary.

Plant oxidases form a class of substances of great importance in plant metabolism, but which are known merely by the reactions they cause, and whose exact nature is quite unknown.

According to Bach and Chodat they form three distinct classes of ferments namely:—

(1) Oxygenases, substances which absorb molecular oxygen forming peroxides.

(2) Peroxidases, which increase the oxidising power of peroxides and can only act in their presence.

(3) Katalases, which destroy peroxides with an evolution of oxygen.

It has long been known that certain of the reactions supposed to characterise oxidase ferments could be produced by certain inorganic metallic salts.* As the result of the detailed investigation of the oxidase action of various metallic salts of copper, iron, chromium, manganese, lead, etc., upon guaiacum, paraphenylenediamine, hydroquinone, pyrogallol, gallic acid, tannic acid, and tyrosin, the conclusion has been formed that the correspondence between the action of organic and of inorganic oxidases is extremely close. It was also found that the oxidase action of a metallic salt varies according to its acid combination, and that in the case of certain salts, such as sodium or potassium ferrocyanide, ferricyanide, phosphate, or chromate, the oxidase action was due to the acid and not to the base. In addition, oxidase action may be accelerated in the presence of sensitisers such as the chlorides or phosphates of sodium or potassium, or retarded or prevented by a variety of antagonisers. The addition of a sensitiser may cause a "peroxidase" to act in the absence of hydrogen peroxide. This applies to both organic and inorganic oxidases, and determinations of the minimal amounts of metallic

* Bertrand, 'Compt. Rend.,' vol. 122, p. 1032 (1896).

oxidases required to produce progressive oxidation in the presence of a sensitiser indicate that their action can be considered as closely akin to that of any enzyme. H. E. and E. F. Armstrong* have shown in a series of valuable papers, and particularly in the hydrolysis of raffinose by acids and enzymes, that a close correspondence exists between the action of organic and inorganic hydrolysing agents. The same appears to hold for organic or inorganic oxidases.

In general, oxidases, whether inorganic or organic, may vary from strong to weak. The former will cause direct oxidation from the oxygen dissolved in a watery solution. The latter will transfer oxygen from labile oxygen compounds such as hydrogen peroxide, or will use dissolved oxygen in the presence of sensitisers such as the chlorides or phosphates of sodium or potassium. Various intermediate grades of activity are shown. There is no reason for separating oxidases and peroxidases as distinct classes of ferments, and peroxides do not necessarily take part in all oxidase actions, although water does. The supposed separation of oxidase and peroxidase by fractional precipitation with alcohol may be merely the result of attenuation.

An oxidase may be a "peroxidase" to certain oxidants or may become so when attenuated. Metallic oxidases act as ferments in that a small amount may produce considerable oxidation, especially in the presence of sensitisers such as salt with copper sulphate, sodium phosphate with potassium ferricyanide, etc., and in that the oxidase appears to act as an intermediary in the chemical change.

Hydrogen peroxide may influence oxidase action:—

(*a*) By providing a supply of labile oxygen.

(*b*) By converting a feeble oxidase into a strong oxidase (ferrous salt into ferric, ferrocyanide into ferricyanide).

(*c*) By acting as a sensitiser to the oxidant substance.

(*d*) By acting as an inhibitor or antagoniser in some cases.

Various salts may act as sensitisers (sodium and potassium chlorides, bromides, and phosphates) or as inhibitors (barium chloride, sodium fluoride, organic or inorganic acids), and in some cases, with increasing concentration, the action of the former is reversed, while a substance which is a sensitiser with one oxidant may act as a reducing agent with another (copper sulphate and salt on indigo carmine).

Strong metallic poisons will arrest the action of organic oxidases or destroy them (apple, potato, carrot, parsnip) if immediate contact or rapid

* 'Roy. Soc. Proc.,' B, vol. 82, p. 349 (1910); vol. 80, p. 312 (1908), etc.

penetration is assured. Hence the organic oxidases are possibly proteids, with or without oxidase metals, in basic or acid combination.

There is no justification for the use of such terms as "peroxidase," "katalase," "œnoxydase," or "tyrosinase," to indicate specific substances, ferments, or groups of ferments. The "tyrosinase" of the potato is also a "katalase," a "peroxidase," a "pyrogallase," a "hydroquinonase," and a "paraphenylendiaminase." It is, however, permissible to use such terms as katalase action or peroxidase action, and such names as laccase, russulase, potatase, carrotase, etc., as temporary names to indicate the origin of the substances, whose chemical nature is yet unknown. Since, however, their oxidase powers will be only one of many properties, it will never be advisable to name them according to these properties alone, any more than it would be in the case of the metallic oxidases. Comparison with metallic oxidases shows that we are not even on safe ground in assuming the existence of specifically distinct classes of plant oxidases, such as phenolases, aminoxidases, and iodoxidases.

The chlorides and phosphates of potassium and sodium are able to act as oxidase sensitisers, and thus may influence special oxidations, or respiration in general. It is possible that they may exert a stimulatory or controlling action on plant metabolism, and that the sodium chloride always present in the ash of plants may not be an entirely useless constituent. This may explain partly why small doses of salt stimulate the growth of many plants, and why phosphates, in addition to being food substances, may act as stimuli to growth. The stimulating action of many metallic salts on growth may be partly due to their oxidase action.

Ursol tartrate turns lignified walls red or reddish-brown. This is not an oxidase reaction, but is an admirable test for lignin, especially valuable for demonstrating the wood elements in pulpy tissue.

Chloroform strongly, and ether more feebly, retard or inhibit katalase action, but they do not suppress oxidase action. After prolonged contact, however, the organic oxidases are slowly attenuated and destroyed.

The liberation of iodine from potassium iodide may be used as a test for the presence of oxidases in living tissues, but does not indicate the existence of any power of producing peroxides. Dried organic oxidases may retain their properties for three weeks or more, and a glycerine extract for five or more months. Where organic oxidases are destroyed by boiling, this is probably the result of proteid coagulation.

The oxidases of the beetroot and potato appear to be related to one another, and to be among the strongest plant oxidases, and the nearest analogies to them are perhaps afforded by ferric salts and ferricyanides. If

the special action of apple oxidase on tannic acid is due to the presence of a phosphatic sensitiser, it would be a feebler oxidase of the same type. Carrot and parsnip oxidases are a grade feebler, but still react to guaiacum in the absence of a peroxide. Malt diastase is still weaker, and papain feebler still, while pepsin may show a weak " peroxidase " reaction with guaiacum, but not any other oxidase action.

The Fixation of Arsenic by the Brain after Intravenous Injections of Salvarsan.

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(Communicated by Prof. W. Bulloch, F.R.S. Received July 8, 1914.)

(From the Bacteriological Laboratory of the London Hospital.)

During the period of probation of salvarsan as an anti-syphilitic remedy, a number of toxic phenomena were reported which led to the belief that this drug had particular neurotropic properties, and was therefore to be used with the greatest circumspection. These fears were very largely founded upon the well known effect of the related drug atoxyl in producing optic atrophy. Subsequent experience has, however, shown that the supposed neurotropic action of salvarsan was due to certain technical errors in its administration.

In 1911 we published (1) an observation which combated the view that salvarsan had neurotropic qualities. We submitted the organs of an infant who died after administration of salvarsan to Dr. W. H. Willcox for analysis, and he reported to us that the brain in this case contained no arsenic, although considerable quantities were present in other organs. We then applied the law of Ehrlich, " corpora non agunt nisi fixata," and argued that, since the brain was free from arsenic, salvarsan could have no neurotropic action.

Exactly similar conclusions were arrived at by Ullmann in 1913 (2). In the course of a very extensive investigation upon the distribution of arsenic in the body after salvarsan injections, he made it quite clear that the brain never contained more than traces of arsenic, and " this fact was evidence against the neurotropic action of salvarsan." Similarly, Morel and